

Vermicompost and farmyard manure effects on biogeochemical performances of *Withania somnifera*

Efectos del vermicompost y estiércol de granja sobre el rendimiento biogeoquímico de *Withania somnifera*

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Abstract

Since time immemorial, village and ethnic communities have been using a medicinal plant, *Withania somnifera* (L.) Dunal. Mainly used as health tonic. Pharmacological activities of this plant include physiologic and metabolic restoration, antiarthritic, antiaging, nerve tonic, cognitive function improvement in geriatric states and recovery from neurogenerative disorders like convulsions, tardive dyskinesia etc. While, the demand of Ashwagandha is increasing, it is mandatory to standardize the cultivation practices. The experiment was laid out in a Randomized Block Design (RBD) with two parameters replicated thrice. As a result, comparative study FYM @ 10t/hac. and vermicompost 1t/hac. (Cow dung + Vegetable wastes + *Eisenia foetida*) recorded double yields. Significantly higher protein was observed under treatment of organic manure viz. 10tonne / hectare with spacing 30 X 45 cm and its morphological study and its biogeochemical study have been done at different stages of plants. Present paper deals with the vermicompost treated and FYM effect on morphological, Chlorophyll, sugar, reducing sugar, protein as well as alkaloids.

Keywords: *Withania somnifera*, VMC, Biogeochemical, FYM.

Resumen

Desde tiempos inmemoriales, las comunidades étnicas han usado a *Withania somnifera* (L.) Dunal, como planta medicinal, principalmente como tónico vigorizante. Las actividades farmacológicas de esta planta incluyen el restablecimiento metabólico y fisiológico, antiartrítico, antienvjecimiento, nervio tónico, mejora la función cognitiva en estados geriátricos y recupera de los trastornos neurodegenerativos como convulsiones, discinesia tardía, etc. Mientras la demanda de "ashwagandha" se va incrementando, se hace necesario estandarizar las prácticas de su cultivo. El experimento fue dispuesto en un Diseño de Bloques al Azar (RBD) con dos parámetros repetidos tres veces. Como resultado el estudio comparativo entre el estiércol de granja (FYM) @ 10t/hac. y el vermicompost 1t/hac. (Estiércol de vaca + desechos vegetales + *Eisenia foetida*) registraron rendimientos dobles. Mayor cantidad de proteína se observó en el tratamiento con desechos orgánicos 10ton/ha. con espaciamientos de 30 X 45 cm. Estudios morfológicos y biogeoquímicos fueron realizados en diferentes estadios de las plantas. El presente estudio muestra los efectos entre el vermicompost y el estiércol de granja (FYM) sobre la morfología, clorofila, azúcar, azúcar reductor, proteínas así como alcaloides en *W. somnifera*.

Keywords: *Withania somnifera*, VMC, Biogeoquímicos, FYM.

Introduction

Withania somnifera (L.) Dunal. "India Ginseng", a plant belonging to the solanaceae family is one of the medicinal plants recommended for cultivation in India, as there is great demand for the plant by the pharmaceutical industries mainly for export (Arpana and Bhagyaraj, 2007). *Withania somnifera*.L. (Dunal), mainly used for health tonic for elderly persons and lactating mothers (Chatterjee et.al, 1995;

Bone, 1996). Pharmacological activities of this plant include physiologic and metabolic restoration, antiarthritic, antiaging, nerve tonic, cognitive function improvement in geriatric states and recovery from neurogenerative disorders like convulsions, tardive dyskinesia (Bhattacharya et. al, 2002; Dhuley, 2000).

The global market of medicinal plant is over 60 billion US \$ per year. India, at present export herbal materials and medicines to the tune of Rs. 446.3 crores,

as against Rs. 20,000 crores from China. The export potential of the country can be raised to about Rs. 3000 crores by the end of the year 2005. An estimated survey indicated that the use of herbal medicine will reach to the tune of three trillion US \$ during 2050, (<http://www.kurzweilai.net>). Currently, WHO encourages, recommends and promotes the inclusion of herbal drugs in national health care programmes, because such drugs are easily available at a reasonable price within the reach of common man and as such are time tested and thus considered to be much safer than the modern synthetic drugs. Significantly higher protein was observed under treatment of organic manure viz. 10tonne /hectare with spacing 30 X 45 cm and its morphological study and its biogeochemical study have been done. Difference between treated and untreated plants has been studied.

Material and Methods

Plant Collection: Wild variety seeds of *Withania somnifera* were collected from Banka district 40 km away from the study area and planted in University Department of Botany, T. M. Bhagalpur University dried for a week at room temperature (25 $\pm$ 2°C) and stored in screw capped bottles under ambient condition before experiment during the following April, 2010. Best quality of seeds have been selected by germination test (Peter, 2000) and Tetrazolium test (Grabe, 1970). Plants were transplanted after three weeks in 30 x 45 cm spacing in field. Experiment was conducted in Post Graduate Botany Department, T.M, Bhagalpur University, Bhagalpur from 2009 to 2010. At the initial stage of flowering and fruiting when active constituents present in high level plants collected from field for morphological and biogeochemical analysis have been experimented.

Morphological and Physical Analysis of Plants: On the basis of best quality physical and chemical profiles of FYM and Vermicompost (Cow dung + Vegetable wastes + *Eisenia foetida*) have been selected for field treatment. Following data were taken 25 plant were taken for each study.

T1= FYM @10 t/hac.

T2 = Vermicompost @ 1 t/hac. (Cow dung + Vegetable wastes + *Eisenia foetida*)

a. Root Length

The experimental plants were up rooted and washed and washed in running water properly. The root lengths were estimated before root samples were stained.

Procedure:

The washed roots were cut into 1cm bits in a petridish containing water. Aliquots of root bits were then taken in a square grid (1cm) Petridish and the number of intersects i.e. points where root bits intersect the grids were counted. The same process was used for the entire root sample.

The total root length was estimated by Tennants (1975) formula:

Total root length (cm) = N/14 x Grid size

Where, N= Number of intersects; 11/14 = Tennant's factor

b. Radius of Root:

The root radius was estimated by slide Caliper. The diameter was measured, five readings were taken which include the minimum as well as maximum diameter region.

c. Surface Area of Root:

The surface area of root was derived by using the general formula:

Surface area of Root = $2\pi rl$

Where, (π = 3.14, r = radius of root, l = length of root)

d. Root Volume

Unrooted plants and roots of the sample plants were washed properly in running tap water without loss of any branch. The washed roots were placed properly on the blotting paper to remove water molecules of surface. The volume of root was taken by immersing the entire root in measuring cylinder full with water. Root volume was represented by ml³.

e. Shoot length

shoot The length of plant was measured by ruler scale. Length of the branches and basal stem length which showed highest length were taken into account. It was derived by following formula:

$$\text{Mean shoot length} = \frac{\text{Length of basal stem} + \text{Total length of all branches of plant}}{\text{Total number of branches taken}}$$

f. Radius of stem:

The surface area of shoot was derived by using

general formula :

$$\text{Surface area of shoot} = 2\pi rl$$

Where, ($\pi = 3.14$, r = radius of root, l = length of root)

g. Area of leaf

Leaf was measured by using Graph paper method. The leaves were clipped and marked on graph paper (10 x 10 mm).

$$\text{Mean leaf area} = \frac{\text{S of total area of total N}^\circ \text{ leaf sample of a plant}}{\text{Total N}^\circ \text{ of leaf of plant}}$$

h. Number of leaves

The total number of leaves of an individual plant was derived by using general formula:

$$\text{Mean number of leaves} = \frac{\text{S total N}^\circ \text{ of leaves of all the replicates}}{\text{Number of replicates}}$$

e. Estimation of Chl a and Chl b, chlorophyll:

The estimation of total chlorophyll content was done by colorimetric method (Yoshida *et. al.* 1976).

f. Estimation of total sugar and reducing sugar

However, for LPO estimation, the tissues were centrifuged in ice-cold potassium chloride (0.15 M) solution. Estimation of total soluble sugar (Dubois

et.al., 1956) and estimation of reducing sugar was done.

g. Estimation of Protein:

The method of Lowry *et al.* (1951) was used for protein estimation. After weighing, the brain tissue were homogenized in 2 ml of ice-cold triple distilled water and sonicated for 15 s. The homogenate was then centrifuged and the supernatant used for the biochemical estimations.

h. Estimation of Phosphorus :

Total phosphorus of different parts of plants samples were estimated by Banik and Dey (1981).

i. Estimation of Alkaloid:

Quantitative analysis of alkaloid was conducted by the Mukherjee (1953).

Result and Discussion

After explanting the test plants, soil sample showed improved level of OC, total N, total P and total K in the range of 148.76%-625.81%, 151.83%-454.93%, and 268.26%-1998.92% respectively over control sample. Following are the landmarks of the present investigation that high drug yielding plants species opening new possibilities of their cultivation.

Table. 1. Effect of Vermicompost on Root growth in *Withania somnifera* (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; *= $p < 0.05$; **= 0.01 ; ***= 0.001 ; NS=Not Significant)

Days	Root length (cm)		Radius of Root (cm/plant)		Volume of Root (ml3)		Surface area of root(cm)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	7.91 $\pm$ 0.22	9.85** $\pm$ 0.28	0.14 $\pm$ 0.02	0.19** $\pm$ 0.03	1.87 $\pm$ 0.22	2.59 * $\pm$ 0.09	7.19 $\pm$ 1.07	11.74* $\pm$ 0.73
45	11.91 $\pm$ 0.18	15.11*** $\pm$ 0.26	0.186 $\pm$ 0.05	0.22 NS $\pm$ 0.02	2.25 $\pm$ 0.09	3.18* $\pm$ 0.31	13.82 $\pm$ 0.52	19.93** $\pm$ 0.64
60	14.35 $\pm$ 0.78	21.82*** $\pm$ 0.46	0.24 $\pm$ 0.02	0.256** $\pm$ 0.08	2.93 $\pm$ 0.17	4.43** $\pm$ 0.21	22.99 $\pm$ 0.23	35.94*** $\pm$ 0.46

Table 2. Effect of Vermicompost on Shoot growth in *Withania somnifera* (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; *= $p < 0.05$; **= 0.01 ; ***= 0.001 ; NS=Not Significant)

Days	Shoot length (cm)		Radius of Shoot (cm/plant)		Surface area of Shoot(cm)	
	T1	T2	T1	T2	T1	T2
30	28.21 $\pm$ 0.77	29.38 NS $\pm$ 0.4	0.14 $\pm$ 0.02	0.16 NS $\pm$ 0.02	24.83 $\pm$ 1.24	29.55* $\pm$ 0.78
45	30.04 $\pm$ 1.49	34.47 * $\pm$ 0.13	0.195 $\pm$ 0.01	0.24 NS $\pm$ 0.03	36.81 $\pm$ 0.57	49.86*** $\pm$ 0.87
60	33.28 $\pm$ 0.03	48.02*** $\pm$ 0.39	0.20 $\pm$ 0.04	0.25 NS $\pm$ 0.07	41.84 $\pm$ 0.87	72.94** $\pm$ 0.55

Table. 3. Effect of Vermicompost on Leaf growth in *Withania somnifera* (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; *= $p < 0.05$; **= 0.01 ; ***= 0.001 ; NS=Not Significant)

Days	N $^\circ$ of leaves/plant		% Increase over control	Leaf Area (cm2)		%Increase over control
	T1	T2		T1	T2	
30	7.00 $\pm$ 0.44	7.67 NS $\pm$ 0.34	9.41	157.71 $\pm$ 1.39	169.91** $\pm$ 6.58	7.75
45	9.67 $\pm$ 1.14	11.67NS $\pm$ 0.88	20.81	261.51 $\pm$ 0.94	390.93*** $\pm$ 0.68	49.51
60	14.67 $\pm$ 1.18	17.02 NS $\pm$ 2.31	16.03	993.12 $\pm$ 0.12	1116.25*** $\pm$ 0.23	19.63

Table 4. Dry matter of *Withania somnifera* at different harvesting period (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; * $p < 0.05$; ** $= 0.01$; *** $= 0.001$; NS=Not Significant)

Days	Leaf (gm)		Stem (gm)		Root (gm)		Total dry matter(gm)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	0.082 $\pm$ 0.04	0.144*** $\pm$ 0.083	0.073 $\pm$ 0.04	0.08 NS $\pm$ 0.04	0.034 $\pm$ 0.018	0.047* $\pm$ 0.048	0.189 $\pm$ 0.11	0.27*** $\pm$ 0.003
45	0.123 $\pm$ 0.06	0.47*** $\pm$ 0.011	0.12 $\pm$ 0.066	0.27*** $\pm$ 0.15	0.98 $\pm$ 0.12	0.097*** $\pm$ 0.05	4.57 $\pm$ 0.19	0.834*** $\pm$ 0.009
60	0.167 $\pm$ 0.09	0.714*** $\pm$ 0.016	0.167 $\pm$ 0.004	0.551*** $\pm$ 0.32	1.09 $\pm$ 0.17	0.199*** $\pm$ 0.11	7.92 $\pm$ 0.17	1.46*** $\pm$ 0.021

Table 5. Chlorophyll content at different harvesting period

Days	Chlorophyll a (μ g/mg)		Chlorophyll b (μ g/mg)		Total Chlorophyll (μ g/mg)		Chi a:Chi b (μ g/mg)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	3.02 $\pm$ 0.09	3.561** $\pm$ 0.08	0.34 $\pm$ 0.012	1.05*** $\pm$ 0.04	3.361 $\pm$ 0.11	4.62*** $\pm$ 0.12	8.87 $\pm$ 0.045	2.31NS $\pm$ 0.98
45	2.36 $\pm$ 0.075	3.923*** $\pm$ 0.03	0.958 $\pm$ 0.038	1.12* $\pm$ 0.07	3.307 $\pm$ 0.064	5.034*** $\pm$ 0.06	2.47 $\pm$ 0.162	3.528** $\pm$ 0.096
60	2.48 $\pm$ 0.043	3.99*** $\pm$ 0.018	1.16 $\pm$ 0.018	0.97NS $\pm$ 0.05	3.64 $\pm$ 0.062	4.96*** $\pm$ 0.063	2.14 $\pm$ 0.006	4.164*** $\pm$ 0.21

Table 6. Free sugar content at different harvesting period

Days	Leaf (μ g/mg)		Stem (μ g/mg)		Root (μ g/mg)		Whole plant (mg/plant)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	2.39 $\pm$ 0.02	2.75NS $\pm$ 0.21	2.512 $\pm$ 0.02	4.87*** $\pm$ 0.11	2.806 $\pm$ 0.21	3.519* $\pm$ 0.15	0.485 $\pm$ 0.015	0.999*** $\pm$ 0.047
45	2.09 $\pm$ 0.14	3.59** $\pm$ 0.17	3.482 $\pm$ 0.08	6.12*** $\pm$ 0.04	4.962 $\pm$ 0.19	5.517* $\pm$ 0.08	1.116 $\pm$ 0.04	4.234*** $\pm$ 0.016
60	3.38 $\pm$ 0.28	6.32*** $\pm$ 0.18	4.24 $\pm$ 0.065	7.43*** $\pm$ 0.23	6.266 $\pm$ 0.14	8.83*** $\pm$ 0.02	1.933 $\pm$ 0.016	10.952*** $\pm$ 0.127

Table 7. Reducing sugar content at different harvesting period

Days	Leaf (μ g/mg)		Stem (μ g/mg)		Root (μ g/mg)		Whole plant (mg/plant)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	0.24 $\pm$ 0.013	0.467*** $\pm$ 0.09	0.24 $\pm$ 0.008	0.754*** $\pm$ 0.04	0.619 $\pm$ 0.027	0.66NS $\pm$ 0.012	0.068 $\pm$ 0.004	0.169*** $\pm$ 0.08
45	0.36* $\pm$ 0.01	0.756*** $\pm$ 0.04	0.305 $\pm$ 0.007	0.84*** $\pm$ 0.008	0.689 $\pm$ 0.07	0.79NS $\pm$ 0.024	0.135 $\pm$ 0.005	0.67*** $\pm$ 0.014
60	0.412 $\pm$ 0.03	0.928*** $\pm$ 0.03	0.412 $\pm$ 0.004	1.197*** $\pm$ 0.06	0.921 $\pm$ 0.014	1.29*** $\pm$ 0.01	0.243 $\pm$ 0.008	1.66*** $\pm$ 0.006

Table 8. Changes in protein content at different harvesting period in *Withania somnifera* (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; * $p < 0.05$; ** $= 0.01$; *** $= 0.001$; NS=Not Significant)

Days	Leaf (μ g/mg)		Stem (μ g/mg)		Root (μ g /mg)		Whole Plant (mg/plant)	
	-VMC	+VMC	-VMC	+VMC	-VMC	+VMC	-VMC	+VMC
30	36.92 $\pm$ 0.057	53.92*** $\pm$ 0.08	17.56 $\pm$ 0.27	18.59* $\pm$ 0.27	17.95 $\pm$ 0.302	23.33*** $\pm$ 0.19	10.83 $\pm$ 0.32	7.506*** $\pm$ 0.27
45	40.996 $\pm$ 0.09	56.39*** $\pm$ 0.58	20.65 $\pm$ 0.24	22.63* $\pm$ 0.51	13.088 $\pm$ 0.014	33.53*** $\pm$ 0.339	17.72 $\pm$ 0.54	39.65*** $\pm$ 0.136
60	42.314 $\pm$ 0.25	63.789*** $\pm$ 0.9	14.24 $\pm$ 0.12	31.07** $\pm$ 0.26	13.774 $\pm$ 0.054	37.97*** $\pm$ 0.09	25.78 $\pm$ 0.095	71.48*** $\pm$ 0.61

Table 9. Phosphate content at different harvesting period

Days	Leaf (μ g/mg)		Stem (μ g/mg)		Root (μ g/mg)		Whole plant (mg/plant)	
	T1	T2	T1	T2	T1	T2	T1	T2
30	0.097 $\pm$ 0.018	0.3762*** $\pm$ 0.013	0.079 $\pm$ 0.046	0.105** $\pm$ 0.06	0.033 $\pm$ 0.002	0.089** $\pm$ 0.007	0.014 $\pm$ 0.002	0.052*** $\pm$ 0.001
45	0.147 $\pm$ 0.04	0.626*** $\pm$ 0.038	0.128 $\pm$ 0.001	0.353*** $\pm$ 0.006	0.062 $\pm$ 0.003	0.199*** $\pm$ 0.003	0.045 $\pm$ 0.026	0.329*** $\pm$ 0.014
60	0.1982 $\pm$ 0.05	1.207*** $\pm$ 0.075	0.179 $\pm$ 0.004	0.721*** $\pm$ 0.009	0.142 $\pm$ 0.008	0.419*** $\pm$ 0.01	0.138 $\pm$ 0.007	1.157*** $\pm$ 0.042

Table 10. Presence of Alkaloid in *Withania somnifera* (- =Absent; + = Moderately presence; ++ = Good presence; +++ = Excellent present)

Leaf	Stem	Root
+	-	+++

Table 11. Alkaloids content at harvesting period in fresh and dry material in *Withania somnifera* (Value of Mean $\pm$ SE of 10 samples; +VMC= Vermicompost; -VMC=Without Vermicompost; * $p < 0.05$; ** $= 0.01$; *** $= 0.001$; NS=Not Significant)

Alkaloid content (mg/plant)		Dry matter (mg/plant)	
-VMC	+VMC	-VMC	+VMC
0.904 $\pm$ 0.005	3.276*a $\pm$ 0.072	0.217	0.225

In present status we found that vermicompost (1 t/hac.) enhances the rate of alkaloid percentage doubles than FYM doses (10 t/hac.) data furnishes morphological effects (table 1-4) also supports chlorophyll content (Table 5), the rate of total sugar (Table 6), reducing sugar (Table 7), phosphate (Table 9), proteins content (Table 8) and alkaloid contents (Table 10 and 11). This might be due the better availability of nutrients from organic and foliar source of nutrients and effective conservation of nutrients such as Fe, Mg and Zn at site of photosynthesis into pigments. The present study has created an interesting data with respect to plant growth, yield characters and biochemical analysis. As evidenced from the work of Xu *et. al.*(2000) and Hartwingon and Evan (2000), this may be due to effective micro-organism enhances the production of phytohormones like auxins and gibberellins that might have stimulated the growth by increasing the plant height, number of branches Humic acid influences plant growth through modifying the physiology of plants and by improving the physical, chemical and biological properties of soil (Dursun *et.al.*, 1999). Humic acid provides carbon as an energy source to nitrogen fixing bacteria and thus proves its biological function (Vaughan, 1974). The natural bioregulator in moringa leaf extract also increased the dry matter production registered increased yield compared to control.

Conclusion

In present study, after explanting the test plants, soil sample showed improved level of OC, total N, total P and total K in the range over FYM sample. Following are the landmarks of the present investigation that high drug yielding plants species opening new possibilities of their cultivation. As FYM dose 10t/hac. and vermicompost dose needs only 1 t/hac. yields doubles registered more effective than FYM dose. Vermicompost treatment better result in Protein, phosphate and sugar content thus, it is economic and easily applicable by nursery workers and poor farmers in developing mass planting stock over costly plant growth regulators and associated technical use in rapid multiplication.

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